Studies on the Phosphatase in Takadiastase

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Table 2. Optial density (Canavanine+Rongalite).

225-300 mμ	Hour of Acetone				
	0	24	48	72	120
225	1.370	—	—	1.628	1.130
230	0.670	1.878	1.840	1.228	0.702
235	0.300	1.106	1.070	0.648	0.420
240	0.125	0.540	0.520	0.334	0.217
245	0.063	0.236	0.220	0.155	0.113
250	0.037	0.096	0.083	0.070	0.065
255	0.022	0.042	0.037	0.039	0.043
260	0.021	0.021	0.022	0.026	0.033
265	0.020	0.015	0.014	0.020	0.026
270	0.021	0.013	0.013	0.017	0.023
275	0.021	0.014	0.014	0.017	0.023
280	0.021	0.015	0.015	0.018	0.022
285	0.021	0.017	0.017	0.018	0.022
290	0.021	0.018	0.018	0.018	0.021
295	0.021	0.020	0.020	0.018	0.020
300	0.021	0.021	0.022	0.019	0.021

The experimental results so far obtained have led the author to the belief in Type I combination.

Therefore, by adding activator such as Rongalite which affect papain molecule, -CHO group is considered to appear, and it is the active group that resolves protein.

21. Studies on the Phosphatase in Takadiastase

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We tried to separate the phosphatase in Takadiastase, using the dialysed Takadiastase solution as an original enzyme solution. As substrate, 0.2 ml. of 1% sodium β-glycerophosphate (GP-ase), 0.5% sodium pyrophosphate (PP-ase) or 0.012 *M* Na_4-ATP (ATP-ase) solution was used. 1) As for the influence of pH of 0.5 *M* acetate or 0.1 *M* veronal buffer solution, the pH curves of GP-ase, PP-ase and ATP-ase activity were all observed to be similar. 2) Among metallic ions, Mg or Ca ion increased, or at least did not reduce, these phosphatase activities, whereas Zn or Mn ion inhibited their activities. 3) When enzyme solutions were kept at 50°, 60°, 70° or 80°C for 10 mins., their activites were strongly damaged at 60°C and completely at 80°C. 4) The original enzyme solution was adsorbed by the same volume of $Al(OH)_3$

C_γ suspension (pH 7) (3% dry weight). The adsorbate was eluted with a mixture of 10% $(NH_4)_2SO_4$ and 3% $NaHCO_3$ solutions. (pH 7.7). It was found that in the eluted solution the activity of the phosphomonoesterase was only 16% of that of the original enzyme solution, whereas the pyrophosphatase activity was 40%. 5) After the adsorption of the enzyme solution by the same volume of $Ca_3(PO_4)_2$-gel (pH 7.0) (5% dry weight), the elution was carried out with a mixture of 10% $(NH_4)_2SO_4$ and 3% $NaHCO_3$ solutions (pH 7.7). The activity of the phosphomonoesterase in the eluate was 20% of the original solution, whereas pyrophosphatase activity was 32%. Among adsorbents examined, kaolin was a little effective, but $Mg(HH)_2$-gel, charcoal or infusorial earth remained to be quite ineffective. 6) A certain quantity of solid $(NH_4)_2SO_4$ was added to the dialysed Takadiastase solution to the partial saturation. Then it was filtered by the hardest filter paper (Toyo Roshi No. 5c), and the precipitate was dissolved in water to make the same volume as the filtrate. In the precipitate, obtained from the solution containing $(NH_4)_2SO_4$ to 60~100% saturation, almost all the activity of the pyrophosphatase or of the phosphomonoesterase remained. 7) The phosphatase activities of the Takaphosphatase were demonstrated to be precipitated by cold acetone in its final concentration of from 33 to 55 volume percent. But the separation of the pyrophosphatase from the other phosphatase was impossible, too. 8) The purification of the pyrophosphatase was performed as follows:- (a) 5g. of Takadiastase was dissolved in 25 ml. of water and dialysed for 40 hours at pH 7.0 (activity ratio being GP-ase : PP-ase=233 : 100). (b) The 1% $Al(OH)_3$ C_γ was added and centrifuged. The precipitate was discarded. (GP-ase:PP-ase=300:100) (c) Then 25 ml. of $Al(OH)_3$ C_γ was further added and centrifuged. The adsorbed enzyme was eluted by 50 ml. mixture of 3% $NaHCO_3$ and 10% $(NH_4)_2SO_4$ solutions. The elute was adjusted to pH 5.0 with CH_3COOH (GP-ase : PP-ase=81 : 100). (d) This eluate was saturated with solid $(NH_4)_2SO_4$ and was filtered by the hardest filter paper. The sediment was dissolved in 10 ml. H_2O and dialysed overnight (GP-ase : PP-ase=67 : 100). (e) This dialysed solution was adsorbed by 10 ml. of $Ca_3(PO_4)_2$-gel and eluted as mentioned above. The enzyme solution was ajusted to pH 5.0 and saturated again with solid $(NH_4)_2SO_4$. The resulted precipitate was dissolved in 10 ml. H_2O and dialysed overnight. This solution showed a strong activity of the pysophosphatase (GP-ase : PP-ase=33 : 100), while the original Takadiastase solution appeared to have a weaker pyrophosphatase activity. (9) The hydrolysis value of ATP and co-carboxylase by this purified enzyme is as follows :

Enzymes	Hydrolysed POH in percent to total-P, when hydrolysis value of pyrophosphatase was regarded as 100%		
	Pyrophosphate	Cocarboxylase	ATP
Original enzyme	100	56	44
Purified enzyme	100	41	25

The results demonstrate that this purified pyrophosphatase might differ from the ATP-ase. But it is not clear whether the enzyme which hydrolyses co-carboxylase is identical with the purified pyrophosphatase or not.

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22. Studies on the Metabolic Products of *Pseudomonas aerginosa* sp. (II)

On the Quantitative Determination of Pyocyanine. (1)

Mamoru KURACHI

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Since any report on the quantitative determination of pyocyanine had not yet been put forward, in the previous investigation amounts of pyocyanine were compared merely by colorimetric observation.

In the present paper, experimental results on photometric determination of pyocyanine by using BECKMAN model DU quartz spectrophotometer are mainly dealt with.

After various attempts, the crystal of blue pigment obtained from cultural solutions of the bacteria was ascertained to be identical with pyocyanine itself by elementary analysis. It was observed that pure crystal was never obtained by repeating recrystallization, whereas blue crystals were easily obtained from concentrated chloroform solution of pyocyanine by adding ethyl ether and then recrystallized from water as very fine needles.

On the other hand, crystals of pyocyanine were successfully obtained from its chloroplatinate ($(C_{13}H_{10}N_2O)_2H_2PtCl_6$.). From the fact mentioned above, it will be suggested that not only destruction of pyocyanine takes place during purification, but its crystal is also contaminated by impurities revealing similar solubility to the solvents.

Besides pyocyanine chloroplatinate, various salts of inorganic acids were obtained. It is noteworthy that with many kinds of organic acids except oxalic acid, pyocyanine salt could not be crystallized. With regard to the absorption spectrum of pyocyanine, several reports have hitherto been put forward, but these results contradicted each other. For the determination of pyocyanine, the author reexamined its absorption spectrum and obtained the following results differed considerably from those of other investigators : with neutral or alkaline pyocyanine solution, absorption maxima are 690 mμ, 379 mμ, 311 mμ and 238 mμ, and minima are 448 mμ, 344 mμ and 270 mμ ; with acid solution, maxima are 520 mμ, 387 mμ, 278 mμ, and 242 mμ, and minima are422 mμ, 318 mμ, 251 mμ and 231 mμ.